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Beta amyloid peptide plaques fail to alter evoked neuronal calcium signals in APP/PS1 Alzheimer's disease mice

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial disorder of unknown etiology. Mechanistically, beta amyloid peptides (Aβ) and elevated Ca^{2+} have been implicated as proximal and likely interactive features of the disease process. We tested the hypothesis that proximity to Aβ plaque might exacerbate activity-dependent neuronal Ca^{2+} signaling in hippocampal pyramidal neurons from $APP_{SWE}/PS1_{M146V}$ mice. Using combined approaches of whole cell patch clamp recording and 2-photon imaging of neuronal Ca^{2+} signals with thioflavin-S plaque labeling in hippocampal slices, we found no correlation between thioflavin-S labeled Aβ plaque proximity and Ca^{2+} responses triggered by ryanodine receptor (RyR) activation or action potentials in either dendrites or somata of AD mice, regardless of age. Baseline and RyR-stimulated spontaneous excitatory postsynaptic potentials also showed little difference in relation to Aβ plaque proximity. Consistent with previous studies, RyR-evoked Ca^{2+} release in $APP_{SWE}/PS1_{M146V}$ mice was greater than in nontransgenic controls. Within the soma, RyR-evoked Ca^{2+} release was elevated in older $APP_{SWE}/PS1_{M146V}$ mice compared with younger $APP_{SWE}/PS1_{M146V}$ mice, but was still independent of plaque proximity. The results indicate that early Ca^{2+} signaling disruptions can become yet more severe with age through mechanisms independent of Aβ plaques, suggesting that alternative pathogenic mechanisms might contribute to AD-associated dysfunction.

1. Introduction

Alzheimer's disease (AD), an age-related neurodegenerative disorder progressing from cognitive dysfunction to death, is associated with multiple risk factors and potential mechanisms (Querfurth and LaFerla, 2010). Considerable research has focused on the neurotoxicity of beta amyloid peptides (Aβ) since they were identified in plaques from AD patients decades ago (Goate and Hardy, 2012; Holtzman et al., 2011; Selkoe, 1993), yet therapeutic studies targeting Aβ have yielded disappointing or counterproductive results (Aisen et al., 2011; Galimberti and Scarpini, 2011; Karran et al., 2011; Selkoe, 2011b; Stone et al., 2011). Among other things, impediments to progress in this arena might reflect uncertainty regarding the relevant Aβ species to target (Benilova et al., 2012), the use of inconsistent model systems or supraphysiologic Aβ peptide concentrations (Castellani and Smith, 2011; Waters, 2010), incomplete understanding of physiologic versus pathologic roles of Aβ and amyloid precursor protein (APP) (Chasseigneaux and Allinquant, 2012; Guo et al., 2012; Zhang et al., 2012), and incomplete consideration of alternative pathogenic mechanisms, particularly those affecting synapses (Chakroborty and Stutzmann, 2011).

Because the mechanisms driving AD pathology appear disparate, effective therapeutic strategies might derive from a confluence of targets that are synergistic with, or alternative to, monopathic Aβ-centric therapies. Ca^{2+} dysregulation is one feature to consider in that it emerges during the aging process (Disterhoft and Oh, 2007; Foster, 2007; Thibault et al., 2007), accelerates Aβ pathology (Demuro et al., 2010; Itkin et al., 2011), and is integral to multiple feed-forward pathologic cascades in AD (Berridge, 2010; Bezprozvanny, 2009; Camandola and Mattson, 2011; Gibson et al., 2010; Hermes et al., 2010; Stutzmann, 2007). Indeed, presenilin mutations associated with familial AD (FAD) cause profound Ca^{2+} signaling exaggerations as one of the earliest, if not the earliest, pathogenic event (Muller et al., 2011a; Stutzmann and Mattson, 2011; Stutzmann et al., 2006; Supnet and Bezprozvanny, 2011) followed by accelerated Aβ deposition when combined with mutant APP (Auffret et al., 2010; Lazarov et al., 2006). In vivo studies in mutant APP and presenilin-1 (PS1) transgenic mouse models have suggested associations between Aβ plaque proximity and abnormal cellular Ca^{2+} in cortex. These include Ca^{2+} influx with synaptic

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hyperactivity (Busche et al., 2008, 2012), triggering of astrocytic Ca^{2+} waves (Kuchibhotla et al., 2009), and elevation of resting Ca^{2+} in neurites (Kuchibhotla et al., 2008). Interestingly, the effect on resting Ca^{2+} was observed in only a fraction of neurites, with ≥70% of neurites within 25 μm of Aβ plaque appearing unaffected (Kuchibhotla et al., 2008). Likely because of the in vivo preparation in these and related studies, evoked and activity-dependent Ca^{2+} responses, electrophysiological membrane properties, synaptic properties, and spiking activity were not investigated. These neurophysiological components are fundamental for neuronal signaling and synaptic plasticity—the cellular mechanisms supporting learning and memory (Berridge, 2010). Indeed, AD mice with early Ca^{2+} signaling abnormalities exhibit marked synaptic deficits and shifts toward synaptic depression before the onset of amyloid deposits (Chakroborty et al., 2012; Goussakov et al., 2010; Palop and Mucke, 2010). Understanding the underlying mechanisms driving early synaptic pathophysiology is key to developing effective therapeutic strategies, because it is the breakdown in synaptic integrity that best correlates with cognitive loss in AD (Scheff and Price, 2006; Selkoe, 2002; Terry et al., 1991).

To accelerate progress in AD therapeutics, it might be productive to regard Ca^{2+} dyshomeostasis as part of a pathologic cycle including Aβ as a component. Here, we considered if close proximity to Aβ plaques can further exacerbate neuronal Ca^{2+} signaling, possibly accounting for the age-related progression of AD as Aβ deposits accumulate. To test this hypothesis, we measured activity-dependent Ca^{2+} signaling and membrane properties in hippocampal CA1 pyramidal neurons and correlated the magnitude of Ca^{2+} responses with distance from Aβ plaques. We used adult $APP_{SWE}/PS1_{M146V}$ mice (APP/PS1), 3–14 months of age, representing the range through which Aβ initially deposits and cognitive deficits develop (Howlett et al., 2004). We found that Aβ plaque proximity did not significantly affect the magnitude of ryanodine receptor (RyR)-mediated Ca^{2+} release or the depolarization-activated Ca^{2+} influx responses, nor did we observe a consistent effect of Aβ plaque proximity on spontaneous synaptic potentials or membrane properties. However, we did observe an age-related increase in RyR-evoked Ca^{2+} release in the somata of APP/PS1 mice. The results suggest that early-onset disruptions of intracellular Ca^{2+} signaling can accelerate with age through mechanisms unrelated to Aβ plaques, and highlight the idea that pathogenic mechanisms other than Aβ might be contributing to AD-associated neuronal pathophysiology and Ca^{2+} dysregulation.

2. Methods

2.1. Animals

$APP_{SWE}/PS1_{M146V}$ transgenic mice were initially obtained from GlaxoSmithKline R&D (Howlett et al., 2004) and subsequently bred in-house. Nontransgenic control mice included J29/C57BL6 bred in-house and C57Bl/6 mice purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Male and female mice between 3 and 14 months old were used. Animals were cared for and used in accordance with protocols approved by the Rosalind Franklin University of Medicine and Science Animal Care and Use Committee.

2.2. Histochemistry

For an overview of Aβ plaque deposits, immunohistochemical and thioflavin-S staining were performed using 40 μm thick sections from paraformaldehyde-fixed brain tissue. Mice were anesthetized with halothane (inhalation) and 8% chloral hydrate (5 mL/kg intraperitoneally) and perfused with cold phosphate-buffered saline (PBS; Invitrogen, Grand Island, NY, USA) followed by 4% paraformaldehyde (5–8 mL at 5 mL/min). The brain was removed, incubated overnight at 4 °C in 30% sucrose (UltraPure; Invitrogen)/4% paraformaldehyde in PBS, and then transferred to 30% sucrose in PBS at 4 °C. Coronal sections were cryostat cut at −23 °C (Thermo Scientific Microm HM 550) and stored at −30 °C in a cryoprotectant mixture of glycerin:ethylene glycol:0.1 M sodium phosphate buffer (pH 7.0): 25:30:50.

Further incubations were conducted at room temperature using a rotary shaker, unless otherwise indicated. For thioflavin-S staining, free-floating sections were washed with Tris-buffered saline (TBS; 0.1 M Tris, 0.9% NaCl, pH 7.5; Sigma-Aldrich) 3 times for 10 minutes, incubated in 0.5% thioflavin-S (Sigma-Aldrich) in 50% ethanol for 10 minutes, and finally washed 2 times in 50% ethanol for 3 minutes followed by double-distilled water for 10 minutes. Stained sections were mounted on glass slides and sealed with 9.6% wt/vol polyvinyl alcohol, 24% wt/vol glycerol, 0.1 M Tris-Cl buffer pH 8.0 (PVA-DABCO; Sigma-Aldrich) under glass coverslips. Slides were dried overnight at room temperature (23 °C) protected from light and stored covered at 4 °C until imaged.

For immunohistochemical staining, free-floating slices were washed in TBS as above, incubated in 70% formic acid for 5 minutes, and then washed in TBS 2 times for 3 minutes each. Slices were blocked by incubation in 5% goat serum for 1 hour (Equitech Bio, Kerrville, TX, USA) plus 0.1% Triton-X 100 (Fluka) in PBS followed by 1 hour in 0.12 mg/mL goat anti-mouse F(ab′)2 (Jackson ImmunoResearch, West Grove, PA, USA). Slices were immunostained by incubation in 1:1000 dilution of 4G8 anti-Aβ 1° antibody (Covance, Madison, WI, USA) in 1% goat serum plus 0.0025% Triton-X 100 in PBS for 72 hours at 4 °C, then washed 3 times in TBS for 10 minutes each, then incubated with 1:1000 goat anti-mouse A488 2° antibody (Invitrogen) in 1% goat serum in TBS for 1 hour, and finally washed in TBS 2 times for 5 minutes each. Stained slices were mounted on glass slides and stored as above. Sections were imaged with a Zeiss 510 confocal microscope with a 10× objective and 1024 × 1024 digital resolution.

2.3. Patch-clamp electrophysiology

The preparation of hippocampal brain slices (300 μm) was modified from methods previously described (Goussakov et al., 2010). Mice were anesthetized with halothane, decapitated, and the brain removed into ice-cold sucrose cutting solution (200 mM sucrose, 1.5 mM KCl, 0.5 mM $CaCl_2$, 4.0 mM $MgCl_2$, 1.0 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM Na-ascorbate (Sigma-Aldrich), and 20 mM dextrose, equilibrated with 95% O_2/5% CO_2). Horizontal hippocampal slices were prepared in a Camden Instruments vibratome with the chamber filled with ice-cold sucrose cutting solution and then transferred to and maintained in standard artificial cerebrospinal fluid (aCSF; 130 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 10 mM dextrose [305–310 mOsm], equilibrated with 95% O_2/5% CO_2, pH 7.3–7.4) at 32 °C for at least 1 hour before use. Before recording, each slice was stained with 0.0005% thioflavin-S in aCSF for 2 minutes at room temperature (23 °C). Patch-clamp recordings were conducted at room temperature (23.5 °C) in continuously superfused aCSF (1.5–2.0 mL/min). Drugs were applied via bath superfusion. Caffeine (20 mM for 1 minute) was used to activate RyR, and although caffeine also has antagonistic effects on the adenosine 1A receptor, we have previously tested and controlled for this (Chakroborty et al., 2009). Patch pipettes (5–7 MΩ) were filled with intracellular solution containing 135 mM K-gluconate, 2.0 mM $MgCl_2$, 4.0 mM Na_2ATP, 0.4 mM Na-GTP, 10 mM Na-phosphocreatine, 10 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) (pH adjusted to 7.3 with KOH; materials from Sigma-Aldrich), and 50 μM